the proHp-stimulated PlGF expression. Transforming growth factor-β (TGF-β) expression and Smad1/5 phosphorylation were also induced by proHp. Blockade of TGF-β signalling by TGF-β receptor kinase inhibitor LY2109761 or Smad1/5 siRNA reduced the proHp-enhanced expressions of PlGF and VEGF-A, and *in vitro* tubular network formation. These findings suggest that the angiogenic effects of proHp were dependent to PlGF and mediated *via* TGF-β/Smad1,5/PlGF/VEGF-A/VEGFR1,2 signalling pathway.

P.09-104-Tue
A draft of the *Hirudo medicinalis* genome provides information about potential anticoagulant and thrombolytic proteins

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Hirudotherapy is used in medicine since ancient times. The salivary cell secretion (SCS) of medicinal leech contains a lot of biological active compounds that suppress blood clotting, decrease pain sensitivity and enhance local blood microcirculation. However, protein and peptide composition of SCS is not fully described, and structure and properties of many components remain unknown. In our work we have generated a draft of the *H. medicinalis* genome. We identified earlier unknown homologs for the genes encoding leech anticoagulants in a draft of medicinal leech genome. There were homologs of serine proteinase inhibitors (bdellin A, bdellin B3, antistasin, eglin C, hirustasin) among identified sequences. We also determined several homologs of destabilase, a polyfunctional protein. Its isopeptidase activity leads to breakdown cross-links in stabilized fibrin and subsequent thrombolysis. This makes destabilase to be a potential compound for treatment of thrombosis. Thus, the draft of the medicinal leech genome provides a database of sequences encoding the unique leech proteins for developing new pharmacological compounds. This work was supported by the Russian Science Foundation (project No. 17-75-20099).

P.09-105-Wed
Analysis of dolichol content in urine and tissues of patients with congenital disorder of glycosylation

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Dolichol (dol) is membrane lipid, which carries glycans for *N*-linked protein glycosylation, *O*-mannosylation and GPI anchor biosynthesis ongoing in endoplasmic reticulum. Its structure is composed by isoprenoid units. Dol is presented in all tissues and most membrane organelles of eukaryotic cells. Recently some types of congenital disorders of glycosylation (CDG) were described as consequence of dol biosynthesis defects (mutations in *DHDDS, SRD5A3, DOLK, NgBR* genes), while other defects are closely associated with its metabolism (genes *MDPU1, DPM1-3*). The aim of study was to analyze dol content in urine and tissues of patients with suspect deficiency in dol biosynthesis by mass spectrometry with purpose to extend screening methods for CDG. Biological material for this study consisted of urine samples from 76 controls in age 2 months to 82 years, 6 patients with CDG (1xNgBR-CDG, 1xSRD5A3-CDG, 2xPMM2-CDG, 1xDPAGT1-CDG, 1xPGM1-CDG) and 43 patients with suspicion of CDG syndrome; samples of frontal cortex, liver, muscle and heart tissues from 2 NgBR-CDG patients and controls. Urine samples were stored in -20°C and tissue homogenates were stored in -80°C until used. Lipid content after extraction was separated by *Agilent 1290 Infinity LC System*. Dols were analyzed by *API 4000 LC-MS/MS System Sciex*. Peaks of dols with 17, 18, 19 and 20 isoprenoid units were captured and the ratio of Dol-18/Dol-19 was calculated. In group of controls, significant correlation between Dol-18/Dol-19 ratio and age in urine was found ($P < 0.005$). Reference range of controls in urine was evaluated. There were not detected differences between genders. Ratio of Dol-18/Dol-19 was significantly increased in NgBR-CDG urine and tissues in comparison with control. Our results showed a new possibility for diagnosis of patients with rare CDG, who cannot be identified by usual screening methods. Supported by: *AZV-16-31932A, RVO-VFN64165, SVV-UK 260367/2017*.

P.09-106-Mon
SGBS preadipocyte cell line can serve for human beige type of thermogenic browning adipocyte differentiation

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In contrast to white adipocytes, brown and beige adipocytes contain high amount of mitochondria which express uncoupling protein 1 (UCP1) and its main function is thermogenesis and energy expenditure. However, there is only limited data about regulatory networks that drive human brown or beige adipocyte differentiation. Therefore, human cell line models are needed in order to explore the key molecular targets of novel pharmacological treatments that can enhance browning and help the therapy of patients suffering from metabolic syndrome. The Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cell line provides a useful tool for studies of human adipocyte biology. Our aims were to investigate whether brown or beige adipocyte differentiation can be induced in SGBS cells and to clarify the effect of Irisin (myokine which stimulates beige differentiation in response to exercise) and BMP7 (autocrine mediator which induces classical brown development) treatment during adipocyte differentiation. We aimed to test the involvement of the creatine phosphate substrate cycle in the heat production of SGBS derived beige adipocytes and investigate whether beige differentiation can be reversed to white adipocytes or they maintain their beige morphology. We applied white and PPARγ-driven browning (including long-term Rosiglitazone treatment) differentiation cocktails to induce adipocyte differentiation. The application of Rosiglitazone could be successfully used to induce browning of SGBS cells. Irisin treatment resulted in up-regulation of UCP1 and TBX1 genes. BMP7 moderately induced a classical brown phenotype. The browning protocol or Irisin induced a beige phenotype with high oxygen consumption rate for UPC1-dependent and creatine phosphate

futile cycle mediated heat production. Thus, SGBS cells can be shifted into both white and beige adipocytes. The continuous Rosiglitazone or Irisin treatment could maintain a beige phenotype under long-term (28 days) differentiation program.

P.09-107-Tue
Protective effect of alpha lipoic acid on apical periodontitis-induced cardiac injury

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Oxygen free radicals are involved in pathophysiology of apical periodontitis. This study was designed to assess the possible protective effect of alpha lipoic acid (ALA) on apical periodontitis (AP)-induced cardiac injury. 200–250 g weighed Wistar albino male rats were randomized into four groups; control group, ALA group, AP group and ALA+AP group. In control and ALA groups, rats were not treated, saline and ALA (100 mg/kg) were administrated. In AP and ALA+AP groups, the left maxillary first molar teeth of the rats were opened with a round dental bur until the pulp chamber was exposed. This application was done under 100 mg/kg ketamine and 10 mg/kg xylazine anaesthesia with using high-speed water cooling. It was then left open for 30 days to induce apical periodontitis. Saline and ALA (100 mg/kg) was administered intraperitoneally every 24 h during the experiment. At the end of the experiment, animals were euthanized by high dose of ketamine-xylazine combination. Serum ALP, LDH, CK and SOD activities were determined using an automated biochemical analyser and the structural cardiac injury was assessed pathologically. Results obtained were then statistically analysed using GraphPad Prism 7. Results were compared by means of one-way analysis of variance (ANOVA). Tukey's was used as a further analysis in binary comparisons. Serum ALP, LDH and CK activities were elevated in AP group. Besides, SOD activities were decreased in the AP group. While the changed enzyme activities was significantly normalized by ALA treatment. Since ALA administration alleviated the apical periodontitis-induced heart injury and improved the cardiac structure and function. It seems likely that ALA with its anti-inflammatory and antioxidant properties may be of potential therapeutic value in protecting the cardiac tissue against systemic oxidative injury due to apical periodontitis.

P.09-108-Wed
ATP6AP1-CDG: Biochemical and molecular-genetic characterization of two cases with severe phenotype

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Introduction: Congenital disorders of glycosylation (CDG) are a rare, clinically heterogeneous group of >100 metabolic diseases caused by deficiencies of enzymes/proteins participating in glycosylation pathways. Here we describe a case of two CDG-suspected male siblings (P1 and P2) with hyperbilirubinemia, hepatopathy, splenomegaly and wrinkled skin, who both died due to progressive liver failure at the age of 3 (P1) and 11 (P2) months. Results and discussion: The finding of hypoglycosylated TF (CDG-II pattern) and ApoC-III in sera from P1 and P2 pointed to a combined defect of N- and O-glycosylation. Altered morphology of Golgi apparatus (GA) and delayed retrograde GA transport, assessed by brefeldin A treatment, was detected by immunocytochemistry in the cultivated fibroblasts of P1. Moreover, peroxisomal disturbance and increased reactive oxygen species were observed, demonstrating a complex impact of the defect on the cellular function. Whole-exome sequencing in P1 identified a novel hemizygous mutation c. 221T>C (p.Leu74Pro) in exon 2 of *ATP6AP1* gene, and the same mutation was later confirmed in P2 by Sanger sequencing, while the mother was found to be a carrier of the heterozygous variant. ATP6AP1 is an accessory protein of the vacuolar H+-ATPase, a proton pump which participates in acidification of various intracellular compartments including GA. ATP6AP1-CDG was first reported in 2016, and compared to the so far 12 described cases presenting predominantly with immunodeficiency, hepatopathy and cognitive impairment, our patients manifested with a more severe phenotype. Supported by grants: AZV MZ CR 16-31932A, RVO-VFN64165, UNCE 204011.

P.09-109-Mon
Hippocampal Ras protein through downstream effectors - Akt and ERK plays a significant role in the nongenomic regulation of thyroid disorders

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Thyroid hormones (THs) are important regulators of growth, development and metabolism. The central nervous system is an important target for the THs and hypothyroidism in adulthood has been clearly linked to neurocognitive dysfunctions. It is also known that THs exert nongenomic effects on the mitochondrial energy metabolism. Nongenomic effects of THs can be mediated by ERK and Akt signaling pathways, and their upstream regulators - Ras proteins. Therefore, we decided to investigate quantitative changes of these signaling molecules in the different compartments of neural cells in the hippocampus of adult rats in following groups: euthyroid (control), hypothyroid (methimazole-treated), nobiletin (nobiletin-treated) and T4-treated hypothyroid states. It was observed that level of phosphorylated ERK was slightly increased in the cytoplasm of hypothyroid rats and significantly decreased in case of nobiletin supplementation. Level of phosphorylated Akt was increased in the cytoplasm of

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